BACKWARD MULTISCATTERING AND TRANSPORT OF PHOTONS IN BIOLOGICAL TISSUE: EXPERIMENT AND SIMULATION

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Abstract. *Optical polarimetry is a mighty tool for study of transparent and translucent inorganic and organic materials. Growing interest in better health and also the quality of the food pointed the investigation of physical properties of biological turbid tissues. Due to the fact that biological tissue is complex random material showing inhomogeneity, anisotropy and nonlinearity in the structure, its rigorous characterization is almost impossible. This complexity also involves an important amount of information. Therefore, the research of polarization states of scattered light is one of emerging novel techniques in biomedical science. The paper deals with the experimental study of degree of polarization and also with simulation of the biological tissue by Monte Carlo method.*

Keywords

Biological and medical application, light transport, Monte Carlo simulations, multiple scattering, polarization, turbid medium.

1. Introduction

One of the important targets of contemporary society is healthy and longer life. So an emphasized interest in advanced biomedical instrumentation to provide more efficient diagnosis, monitoring, and treatment of people is foreseen [1], [2]. At present, optical measurement methods are the powerful tools for basic and applied research and inspection of the characteristic properties of a variety of materials, especially following the development of lasers and computers. In this context, new applications are developed by emerging technologies in lasers, optoelectronic devices, fiber optics, physical and chemical sensors, and imaging [3].

2. Biological Tissue Aging

Living tissue is made up of cells. There are many different types of cells, but all have the same basic structure. Tissues are layers of similar cells that perform a specific function. Among four basic types of tissue – connective, epithelial, nerve, and muscle ones – the latter one is best for investigation and measurement of the aging process [4], [5]. More precisely, among three types of muscles tissues – striated, smooth and cardiac – only first ones that move the skeleton are appropriated for this kind of investigation due to their fibrous nature.

Due to the fact that biological tissue is complex random material showing inhomogeneity, anisotropy and nonlinearity in the structure, its rigorous characterization is almost impossible. On the other hand, this complexity involves an important amount of information. Therefore, the research of polarization states of scattered light is one of emerging novel techniques in biomedical science and food control.

All vital biological organs begin to lose some functions with age. One can find aging changes in body cells, tissues and organs, and these changes affect the functioning of whole body system [6].

3. Polarization and Scattering

Light scattering in biological tissues originates from the tissue inhomogeneities such as cellular organelles, extracellular matrix, blood vessels, etc [7]. This often translates into unique angular, polarization, and spectroscopic features of scattered light emerging from tissue [3]. Hence information about tissue macroscopic and microscopic structure can be obtained from the characteristics of scattered light.

Multiple scattering of light leads to the loss of initial polarization, phase, coherence and wavefront of incident optical radiation [8]. Multiple forward and

backward scattering in biological tissues results in depolarization which is the most prominent polarimetric effect. It is caused by the high density of tissue scattering centers, originating from the random fluctuations of the local refractive index in the tissue microstructure (inside the cell and in the extra-cellular matrix). In fact, the tissue scattering centers vary in size (and shape) from micrometer scale and below to several tens of micrometers. Typical refractive index fluctuations in these scattering structures vary from n_s ~1,4–1,5 (the average background refractive index of cytoplasm and interstitial fluid n_m ~ 1,34). Light scattering from all of these microscopic scattering structures contributes in a complex fashion to the observed depolarization of light in the tissue.

4. Stokes Vector and Mueller Matrix

A scatterer changes the state of the incoming polarized light by mixing the initial polarization states of the incident electric field vectors $E_{\|0}$, and $E_{\perp 0}$. Here $E_{\|0}$, and $E_{\perp 0}$ are the initial components parallel and perpendicular to the scattering plane, respectively. The new parallel and perpendicular electric field components $E_{\|}$ and $E_{\perp}$ arise through an interaction represented by mixing coefficients A_i.

$$\begin{vmatrix} E_{//} \\ E_{\perp} \end{vmatrix} = \begin{bmatrix} A_2 & A_3 \\ A_4 & A_1 \end{bmatrix} \begin{bmatrix} E_{//0} \\ E_{\perp 0} \end{bmatrix}. \tag{1}$$

The Stokes vector **S**, which completely characterizes the intensity and polarization of a light ray, is defined by

$$\begin{vmatrix} I \\ Q \\ U \\ V \end{vmatrix} = \begin{vmatrix} \langle E_{//}E_{//}^* + E_{\perp}E_{\perp}^* \rangle \\ \langle E_{//}E_{//}^* - E_{\perp}E_{\perp}^* \rangle \\ \langle E_{//}E_{\perp}^* + E_{\perp}E_{//}^* \rangle \\ \langle i(E_{//}E_{\perp}^* - E_{\perp}E_{//}^*) \rangle \end{vmatrix} = \mathbf{S}, \tag{2}$$

where I, Q, U, and V are Stokes vector elements. I is the total detected light intensity which corresponds to addition of the two orthogonal component intensities, Q is a difference of intensity of polarization at 0 ° or 90 ° to the scattering plane, U is the intensity that corresponds to the difference between intensities of linear +45 ° and −45 ° polarization states, and V is a difference between intensities of a right circular and left circular polarization states.

The general scattering matrix given above applies to any system of particles. However, it is convenient to divide the particles into two rather broad ranges depending on the ratio of wavelength λ to particle size d. The two classes of scattering particles are called Rayleigh and Mie particles.

Particles smaller than the wavelength λ of the incident radiation are called "small particles" or Rayleigh particles. For this condition ($d << \lambda$) the scattering matrix can be exactly calculated. It has the form:

$$[S] = \text{constant} \begin{bmatrix} (1+\cos^2\theta) & \sin^2\theta & 0 & 0 \\ \sin^2\theta & (1+\cos^2\theta) & 0 & 0 \\ 0 & 0 & \cos\theta & 0 \\ 0 & 0 & 0 & \cos\theta \end{bmatrix}. \tag{3}$$

In the Stokes formalism, the following polarization parameters of any light beam are defined [3]:

- Net degree of polarization

$$\text{DOP} = \frac{\sqrt{Q^2+U^2+V^2}}{I}, \tag{4}$$

- degree of linear polarization

$$\text{DOP - L} = \frac{\sqrt{Q^2+U^2}}{I}, \tag{5}$$

- degree of circular polarization

$$\text{DOP - C} = \frac{V}{I}. \tag{6}$$

While the Stokes vectors represent the polarization state of light, a 4×4 matrix **M,** known as the Mueller matrix describes the transfer function of any medium in its interaction with polarized light [9]

$$\mathbf{S_0} = \mathbf{M} \cdot \mathbf{S_i}. \tag{7}$$

Si and **So** being the Stokes vectors of the input and output light, respectively. The 4×4 real Mueller matrix **M** possesses at most 16 independent parameters (or 15 if the absolute intensity is excluded), including depolarization information.

All the medium polarization properties are encoded in the various elements of the Mueller matrix, which can thus be thought of as the complete "optical polarization fingerprint" of a sample. The fundamental requirement that real Mueller matrices must meet is that they map physical incident Stokes vectors into physically resultant Stokes vectors. Although both the Jones and Stokes–Mueller approaches rely on linear algebra and matrix formalisms, they are different in many aspects. Specifically, the Stokes–Mueller formalism has certain advantages. First of all, it can encompass any polarization state of light, whether it is natural, totally, or partially polarized (can thus deal with both polarizing and depolarizing optical systems). Secondly, the Stokes vectors and Mueller matrices can be measured with relative ease using intensity-measuring conventional (square-law detector) instruments, including most polarimeters, radiometers, and spectrometers.

Since biological tissue is a turbid medium where significant depolarization is encountered due to strong multiple scattering effects, the Stokes–Mueller formalism has been used in most tissue polarimetry applications. In contrast, the use of the Jones formalism has been limited

as a complementary theoretical approach to the Mueller matrix calculus, or to studies in clear media, specular reflections, and thin films where polarization loss is not an issue. In this paper, we review the use of the Stokes–Mueller approach for noninvasive assessment of biological tissues, discuss inverse analysis methods for extraction/quantification of the intrinsic tissue polarimetry characteristics, and provide selected illustrative application examples of tissue polarimetry. In the following, we define the basic medium polarization properties through the Mueller matrix formalism.

5. Experimental

5.1. Experimental Set-Up

There are several experimental systems capable of detecting weak polarization signals in the presence of large diffusive background for light scattered from a random biological medium [10]. For our purpose, the best setup for back-scattered polarized light measurement is depicted in Fig. 1.

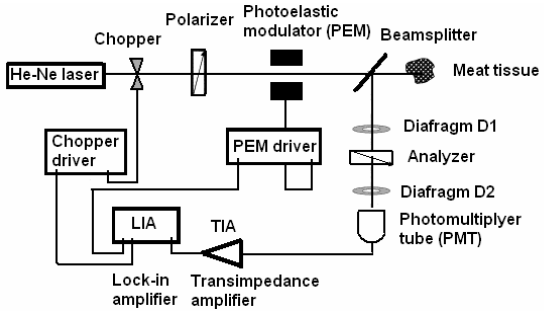

Fig. 1: Schematic of the experimental system for measuring polarization properties of back-scattered diffusely reflected light from a turbid biological sample [9].

Its optical part consists of a He–Ne laser source (λ = 632,8 nm), a linear polarizer, a 50 kHz photoelastic modulator (PEM) to enable time-varying polarization states to impinge on the beam splitter and then onto the turbid sample, and an analyzer-photodetector combination to measure the scattered light intensity and the polarization properties of the scattered light. Electrical part then consists of the lock-in and transimpedance amplifiers enable sensitive synchronous measurement of the resultant photocurrent. A mechanical chopper in the optical path operating at ~ 150 Hz is also used; when tuned to its rotation frequency, the lock-in measures the overall light intensity, and when tuned to the PEM's oscillation frequency (and its harmonics), the lock-in signal is sensitive to the polarization fraction that has survived the sample interactions. The beam splitter is present to enable detection of light that emerges from the sample centered on the exact backscattering direction. The polarizer is at 45 ° with respect to the plane of the optical table, and the orientation of the analyzer is set to rotate in the 0–360 ° range. D1 and D2 are pinhole diaphragms.

5.2. Samples

The experiments were carried out on a population of 15 samples (2×2) cm^2 obtained from chicken breast muscles. Each muscle was cut in slices of 1-15 mm thick and fixed on the mirror or nontransparent background plate for the backscattered measurement. The slices of meat were cut in parallel or perpendicular directions to the muscle fibers. Therefore, the light has passed the optical path twice in the sample, therefore we obtained thicknesses from 2 to 30 mm.

6. Monte Carlo Method

The formal theory of elastic light scattering deals with Maxwell's equations, boundary conditions, and idealized physical models for the scatterer, with matrix algebra, which manipulates the interaction matrices, and with light vectors, which describe the optical system, ignoring the exact mechanism causing the scattering [11]. The fundamental problem of deriving structural features of the scatterer from scattering information is presently unsolvable for most biophysical cases. However, the matrix algebra can be used to catalog the scattering signals, which are related to the matrix elements involved in the scattering process.

Different Monte Carlo (MC) simulation methods, as applied to the transport of light radiation, are principally based on the radiation transfer equation (RTE) and involve computer-simulated calculations of photon propagation in scattering media (e.g. [12], [13], [14]). Simulation of the photon trajectories in Monte Carlo method generally consists of the following key stages:

- injection of the photon in the medium, generation of the photon path-length,
- generation of a scattering event,
- definition of reflection/refraction at the medium boundaries,
- definition of detection,
- accounting for the absorption.

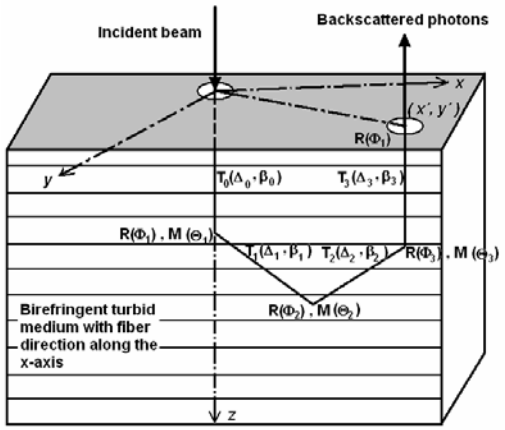

Fig. 2: Geometry of a multiple scattering event in a linearly birefringent turbid medium.

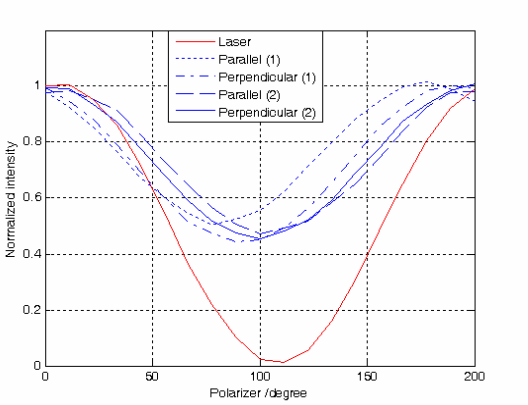

Fig. 3: Angular dependence of polarization directions of backscattered light for two slices of meat sample: 10 mm (curves 1), and 15 mm (curves 2) along the muscle fibers and orthogonally to them.

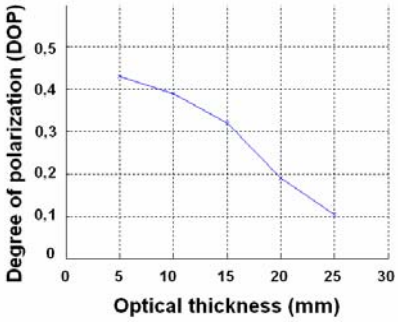

Fig. 4: Dependence of degree of polarization (DOP) on a thickness of chicken slices.

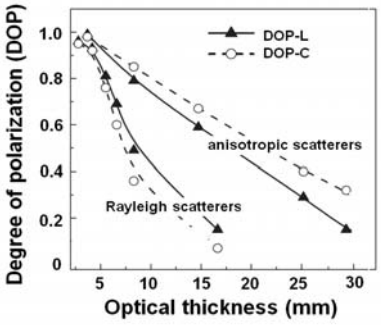

Fig. 5: MC calculated dependence of DOP-L for linear polarization and DOP-C for circular polarization of light vs. optical thickness for anisotropic phantom scatterers.

7. Results

The result of experimental measurement provided on aged sample (Figs. 3 and 4) and result of simulation for anisotropic circular scatterers and Rayleigh spherical scatterers made from polystyrene spheres are presented in Fig. 5.

Red line in Fig. 3 represents normalized intensity given by Malus law only with crossed polarizers, without sample. Blue lines indicate related intensities of backscattered light for two slices of meat: 10 mm (curves 1), and 15 mm (curves 2) along the muscle fibers and orthogonally to them. Figure 4 shows how polarization states change after multiple backward reflections, where initial linearly polarized laser light is modulated by object, and circular and elliptical polarization emerge on the detector. As a result a degree of polarization vs. thickness of meat slides is provided.

If the use of polarized light is also taken into account, the description of polarization states of photons must be included in the flow chart of Monte Carlo program (Fig. 1). To compare results of simulation with experimental results, the phantom medium consisting of tiny polystyrene spheres was considered (Fig. 5).

Figure 5 compares a dependence of degree of polarization (DOP) calculated for linear polarization (DOP-L) (Eq. (5)) and DOP-C (Eq. (6)) for circular polarization of light vs. optical thickness for anisotropic phantom scatterers medium – same number of polystyrene spheres (diameter of 200 nm) diluted in water in cuvette of different lengths. The same measurements for Rayleigh scattering on polystyrene spheres (d = 60 nm) have also been provided. Circular polarization survives more scattering events than the linear one for larger particles, whereas for smaller ones the effect is inversely proportional.

8. Conclusion

Biomedical polarimetry research has two major directions, tissue imaging and tissue characterization. Polarization can be used as an effective tool to discriminate against multiply scattered light and thus can facilitate higher resolution imaging of tissue and its underlying structure. Moreover, the intrinsic polarimetry characteristics themselves contain a wealth of

morphological, biochemical, and functional information that can be exploited for noninvasive and quantitative tissue diagnosis. For either of these applications, accurate measurement of the polarization retaining signal is extremely important.

Stokes polarimetry could replace many of the traditional polarimetric systems which are not suitable for biological tissue examination (e.g., crossed linear polarizers used in microscopy for examining thin fixed *ex vivo* tissue slices). This follows because multiple scattering in thick tissues leads to depolarization of light, creating a large depolarized source of noise that hinders the detection of the small remaining information-carrying polarization signal. A variety of experimental tools have therefore been developed to maximize measurement sensitivity, so that reliable measurements and analyses of the tissue polarimetry data can be performed.

The proposed method can be employed to perform measurement of both Stokes vectors of the light upon interacting with the sample, and/or of the Mueller matrix of the sample itself. Moreover, a comparison of measured and calculated DOP (Fig. 4 and 5) provides a good agreement of results.

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